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Four new species of *Georissa* W. Blanford, 1864 (Gastropoda, Hydrocenidae) from Thailand

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Abstract

Hydrocenid snails have received little research attention compared to other land snail groups, arguably due to their small size. The present study examines the diversity of hydrocenid snails in limestone hills in central, eastern and southern Thailand. Four new species of *Georissa* W. Blanford, 1864 are described: *G. quinquelirata* **sp. nov.** from central Thailand, *G. sagitta* **sp. nov.** and *G. kohsichangensis* **sp. nov.** from eastern Thailand, and *G. digitinota* **sp. nov.** from southern Thailand. These four new species are distinguished by unique characteristics in their shell, including protoconch sculpture and shell shape, operculum, and radula morphology. A phylogenetic analysis of mitochondrial DNA sequences confirms the distinctiveness of all four species.

Key Words

conchological, Neritimorpha, Southeast Asia, systematics, terrestrial snails

Introduction

Microscopic land snails of the family Hydrocenidae Troschel, 1857 are widely distributed with a range encompassing various Pacific islands, New Zealand, Australia, the Indo-Australian Archipelago, parts of Europe, Asia, Madagascar, and Africa (Egorov 2005). In Asia, hydrocenid snails have been documented in Thailand, Cambodia, Laos, Myanmar, Vietnam, Malaysia, Japan, the Philippines, India, and China (Panha and Burch 2005). Traditionally, the systematic taxonomy of hydrocenids rests primarily on morphological characters. Overall, Hydrocenidae have received little attention in research due to their tiny shell size while the delineation of species is complicated by considerable conchological variation in some species in response to diverse ecological conditions in the their habitats. DNA barcoding holds significant potential to identify cryptic species thereby overcoming the limitations of traditional morphological characters in species identification and delimitation in these micro-snails. DNA barcoding has been useful in identifying a broad spectrum of micro snails, such as in the ellobiids *Zospeum* and *Carychium* (Weigand et al. 2011, 2013), the diplommatinid *Plectostoma concinnum*, the cyclophorid *Alycaeus jagori* (Hendriks et al. 2019), and the gastroscopic *Hypselostoma latispira* (Lipae et al. 2020). However, morphological analyses remain the primary method for distinguishing species despite the challenges posed by the tiny size, conchological variability, and ecological plasticity of hydrocenids. Hence, a combination of molecular and morphology analyses promises to be the best approach for identifying different species within this group.

Georissa is a genus of operculated micro-land snails frequently found in limestone environments. The majority of *Georissa* species have so far been recorded in Malaysia (Schilthuizen et al. 2005, 2012; Haase and Schilthuizen 2007; Phung et al. 2017; Khalik et al. 2018, 2019a, 2019b). Notably, DNA barcoding has proven effective in distinguishing *Georissa* species in Malaysia, as demonstrated in the studies of Khalik et al. (2018, 2019b). These studies reported thirteen scaly species and sixteen non-scaly species from Malaysian Borneo alone.

The cytochrome c oxidase subunit 1 (COI or COX1) gene within mitochondrial DNA served as the predominant and widely utilized DNA barcoding marker in animals, proving to be effective for identification purposes in *Georissa* (Khalik et al. 2018, 2019a, 2019b; Hendriks et al. 2019) and were used in this study. However, other genes such as 16S ribosomal deoxyribonucleic acid (rDNA), 18S rDNA, 28S rDNA, histone 3 (H3) and the aquaporin gene have also been analyzed and successfully delineated in *Georissa* (Kano et al. 2003; Schilthuizen et al. 2005, 2012; Uribe et al. 2016; Colgan and Santos 2018; Khalik et al. 2018, 2019a, 2019b; Hendriks et al. 2019). However, DNA barcoding has not been conducted on *Georissa* in China, Cambodia, Laos, Myanmar, Vietnam, and Thailand. A list of *Georissa* species and their distribution on the mainland in Southeast Asia and China is given in Suppl. material 1.

In Thailand, six species and two subspecies of the genus *Georissa* have been identified (BEDO 2017) and reported through morphological analysis: *G. illex* Benson, 1856, *G. blanfordiana* Stoliczka, 1871, *G. liratula* Stoliczka, 1871, *G. monterosatiana monterosatiana* Godwin-Austin & Nevill, 1879, *G. semisculpta* Godwin-Austen & Nevill, 1879, *G. williamsi* Godwin-Austen, 1889, *G. monterosatiana samuiana* Möllendorff, 1894 (Benson 1856; Stoliczka 1871; Godwin-Austen and Nevill 1879; Godwin-Austen 1889; Möllendorff 1894). This research aimed to present the findings on four new species of hydrocenid snails from Thailand, identified as novel to science based on their shell, operculum, and radula morphology, incorporating DNA barcoding.

Materials and methods

Specimen sampling

The specimens have been received from Asst. Prof. Pongrat Dumrongrojwattana and were collected during fieldwork between July 2019 and February 2023. We surveyed four locations, including Pathawi limestone hill, Uthai Thani Province (15°28'26.9"N, 99°45'25.0"E), Yai Man Cave, Chonburi Province (13°09'08.2"N, 100°48'28.0"E), Khao Maka cave temple, Sa Kaeo Province (13°47'16.6"N, 101°56'53.7"E), and Khao Noi Phothiyan temple, Satun Province (06°45'24.2"N, 100°01'54.2"E) (refer to map in Fig. 1). Hydrocenid specimens were collected through visual searching. We collected both living individuals and empty shells. When collecting empty shells, we picked the operculum from the rock surface to which the shell was attached or from the soil where shells accumulated. Living snails were drowned in water and subsequently preserved in 50% ethanol. These procedures were carried out in the Biology Department, School of Science, at King Mongkut's Institute of Technology Ladkrabang (KMITL) and the Biology Department, Faculty of Science, at Burapha University (BUU).

Morphological studies

The collected specimens were thoroughly cleaned and air-dried. Shell was digitally photographed in standardized views (Callomon 2019). We measured the following shell dimensions (in mm): Shell height (SH), shell width (SW), aperture height (AH), and aperture width (AW) on photographs by using ImageJ version 1.8.0. (Schneider et al. 2012). Taxonomic identifications were carried out by referencing the works of Vermeulen and Whitten (1998) and Panha and Burch (2005). To extract the radula, snails were boiled in 1% (w/v) sodium hydroxide (NaOH) solution in a test tube for 10–20 minutes depending on sample size (modified from Geiger et al. 2007). Subsequently, the radula was extracted under a stereo microscope by using an insect pin No. 0, rinsed with distilled water, and dehydrated in a series of ethanol concentrations (increasing from 10, 30, 50 to 70% for 5 minutes each concentration) (Dumrongrojwattana and Tanmuangpak 2020). Shells, opercula, and radulae were imaged under a scanning electron microscope (SEM). Type specimens were deposited in the Zoological Research Collection of Burapha University (ZRCBUU) in Chon Buri Province, Thailand.

Molecular analysis

Genomic DNA was extracted from representative individuals of different morphotypes by using the GF-1 tissue DNA extraction kit. Preserved snails were rinsed twice in sterilized distilled water before extraction. Partial sequences of the COI gene were amplified by Polymerase Chain Reaction (PCR) using primers the LCO1490 and HCO2198 (Folmer et al. 1994). For PCR protocols and conditions refer to Khalik et al. (2019a). Gel electrophoresis with 1% agarose gel was employed to evaluate the quality and size of the PCR products. DNA sequencing was performed using the Barcode Tag Sequencing (BTSeq) technique, which relies on Next-Generation Sequencing (NGS), at Celemics, Inc., Korea. Twenty-nine sequences were analyzed in this study, including 21 sequences sourced from GenBank and eight from this study. All sequences have been deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank) (Table 1). Bayesian Inference was employed to reconstruct a phylogenetic tree using MrBayes version 3.2.7. (Ronquist et al. 2012). The Effective Sample Size (ESS) values and log-likelihoods were monitored to ensure the analysis reached stationarity. Model selection for the COI gene phylogenetic tree was performed using jModelTest 2 (Darriba et al. 2012), based on the Bayesian Information Criterion (BIC). The Bayesian Inference analysis was conducted with parameters set to 10,000,000 generations, sampling every 1,000 generations, and discarding the first 25% of trees as burn-in. (Pholyotha et al. 2021).

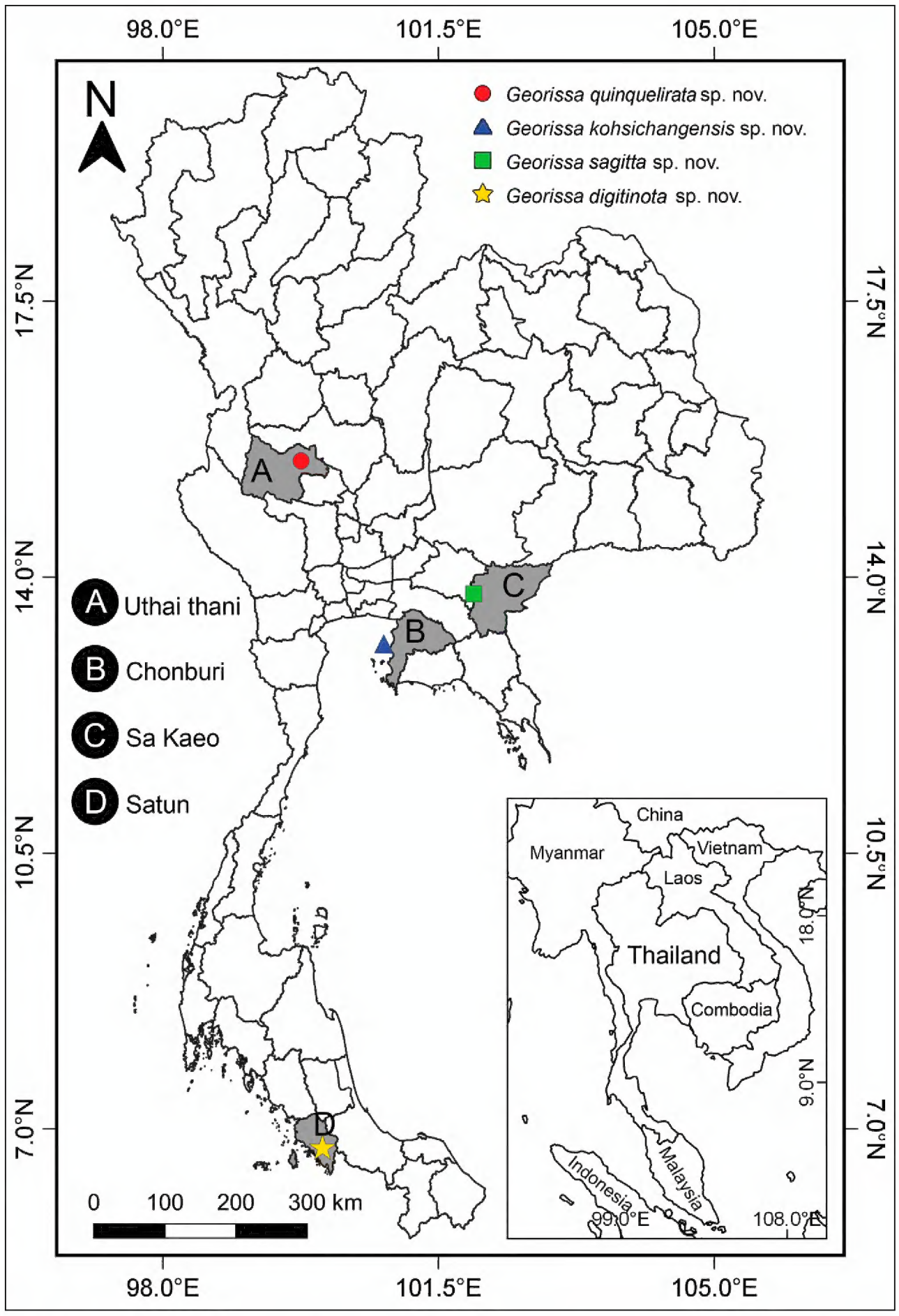

Figure 1. Type localities of *Georissa quinquelirata* sp. nov. (marked with a red circle) in central Thailand, *Georissa kohsichangensis* sp. nov. (marked with a blue triangle), and *Georissa sagitta* sp. nov. (marked with a green square) in eastern Thailand, along with *Georissa digitinota* sp. nov. (marked with a yellow star) in southern Thailand.

Results

Specimen sampling and morphological studies

Hydrocenid snails were found outside the cave, on limestone surfaces, and were particularly abundant on moist surfaces. Based on visual inspection, the shells are tiny, with an average height ranging from 1 to 3 mm. Living snails were orange to brownish. By contrast, deceased snails were white to pale yellow. The shell shape is uniformly round, with spiral cords on the body whorl, the number of which varies among species. Additionally, the aperture is typically round to ovate, the umbilicus is closed, and the white chitinous (corneous) operculum is thin and opaque. Through morphological examination and DNA barcoding, four new *Georissa* species in Thailand were identified, including *G. quinquelirata* sp. nov. from Uthai Thani Province, *G. kohsichangensis* sp. nov. from Chonburi Province, *G. sagitta* sp. nov. from Sa Kaeo Province and *G. digitinota* sp. nov. from Satun Province. The type localities of four new *Georissa* species are displayed in Fig. 1.

Each of the four new *Georissa* species exhibits distinct shell characteristics by first classifying groups based on shell shape. Then, species within each group are distinguished by the number of shell whorls. Further differentiation is made based on shell size for species with the same number of whorls (W). Additionally, the sculpture

Table 1. Sequences used in this study.

No.	Species	Accession number	Reference
1	*Georissa bauensis* isolate B.002	MH033937	Khalik et al. 2018
2	*Georissa bauensis* isolate Q.006	MH033943	Khalik et al. 2018
3	*Georissa hadra* isolate LC.01	MH033896	Khalik et al. 2018
4	*Georissa hadra* isolate LC.03	MH033894	Khalik et al. 2018
5	*Georissa hungerfordi* voucher H.002	MK505430	Khalik et al. 2019b
6	*Georissa hungerfordi* voucher I.002	MK505438	Khalik et al. 2019b
7	*Georissa kinabatanganensis* isolate K.002	MH033958	Khalik et al. 2018
8	*Georissa kinabatanganensis* isolate K.005	MH033960	Khalik et al. 2018
9	*Georissa muluensis* isolate LGG.01	MH033893	Khalik et al. 2018
10	*Georissa muluensis* isolate LGG.03	MH033892	Khalik et al. 2018
11	*Georissa nephrostoma* voucher Knep.001	MK505439	Khalik et al. 2019b
12	*Georissa niahensis* isolate GC.02	MH033880	Khalik et al. 2018
13	*Georissa niahensis* isolate PC.04	MH033954	Khalik et al. 2018
14	*Georissa pachysoma* voucher BSM2.03	MK505441	Khalik et al. 2019b
15	*Georissa pachysoma* voucher BSM2.04	MK505440	Khalik et al. 2019b
16	*Georissa sepulutensis* isolate Sca.002	MH033964	Khalik et al. 2018
17	*Georissa sepulutensis* isolate Sca.004	MH033953	Khalik et al. 2018
18	*Georissa silaburensis* isolate SIG3.03	MH033948	Khalik et al. 2018
19	*Georissa silaburensis* voucher SIG4.04	MK811541	Khalik et al. 2019a
20	*Diplommatina centralis* isolate 4050	HM753339	Webster et al. 2012
21	*Plectostoma concinnum* voucher KPH01588.25	MH254006	Hendriks et al. 2019
22	*Georissa quinquelirata* sp. nov. isolate CPW01.01	PP844569	This study
23	*Georissa quinquelirata* sp. nov. isolate CPW01.03	PP844570	This study
24	*Georissa kohsichangensis* isolate EYM01.03	PP844571	This study
25	*Georissa kohsichangensis* isolate EYM01.04	PP844572	This study
26	*Georissa sagitta* isolate EMK01.01	PP844573	This study
27	*Georissa sagitta* isolate EMK01.02	PP844574	This study
28	*Georissa digitinota* sp. nov. isolate SKN01.04	PP844575	This study
29	*Georissa digitinota* sp. nov. isolate SKN01.05	PP844576	This study

pattern and number of lines on the last body whorl, as well as the shape and surface of the operculum, are used for grouping. These characteristics examined under a stereo microscope, and SEM images were utilized (Figs 2, 4). The shell shape can be categorized into two types: *Georissa sagitta* sp. nov., *G. quinquelirata* sp. nov., and *G. kohsichangensis* sp. nov. have an ovate-conical shape. In particular, *G. kohsichangensis* sp. nov. reveals slight variation whereby the upper half part of the body whorls slopes slightly downward. Conversely, *G. digitinota* sp. nov. has a globose-conical shape (Fig. 2). The species are listed in the order from largest to smallest size as follows: *G. sagitta* sp. nov. (2.4–2.9 mm), *G. digitinota* sp. nov. (2.3–2.7 mm), *G. quinquelirata* sp. nov. (1.9–2.5 mm), and *G. kohsichangensis* sp. nov. (1.7–2.1 mm). Based on the sculpture pattern and the number of lines on the last (or body) whorl, *G. quinquelirata* sp. nov. features a carina-style sculpture with 5–6 prominent cords and weak growth lines between the carinae (Fig. 4A). *Georissa kohsichangensis* sp. nov. and *G. sagitta* sp. nov. exhibit a lirae-style sculpture, with *G. kohsichangensis* having 6–7 cords (Fig. 4E) and *G. sagitta* having 8–9 cords (Fig. 4I). In contrast, *G. digitinota* sp. nov. displays more than 30 prominent striae or spiral lines, which are more frequent than those of the other new species (Fig. 4M). Each new species examined shows a well-incised suture. The protoconch of each species (Fig. 4B, F, J, N) exhibits distinct microscopic sculpture patterns. The microscopic sculpture of the protoconch is wrinkled in *G. quinquelirata* sp. nov. (Fig. 4C), perforated in *G. kohsichangensis* sp. nov. (Fig. 4G), a combination of rounded, ellipsoidal, irregular sculptures and oval indentations in *G. sagitta* sp. nov. (Fig. 4K). In contrast, *G. digitinota* sp. nov. presents an ellipsoidal to irregular sculpture with an oval fingerprint-like indentation (Fig. 4O). All species assessed display a closed umbilicus. The aperture of the shell is observed; *G. quinquelirata* sp. nov., *G. sagitta* sp. nov., and *G. digitinota* sp. nov. are semi-rounded shape while *G. kohsichangensis* sp. nov. is rhombate-shaped. The operculum is paucispiral, featuring a smooth outer with an apophysis, and an arched peg near the base on the inner side the base (Fig. 4D, L, P, H). Regarding the shape of the operculum, *G. quinquelirata* sp. nov., *G. sagitta* sp. nov., and *G. digitinota* sp. nov. display a semi-rounded shape (Fig. 4D, L, P), whereas *G. kohsichangensis* sp. nov. features a rhombate shape (Fig. 4H).

The radula of all examined new species is rhipidoglossate; revealing two long spiral teeth attached to a thin radula plate in the central teeth area on the radula overview (Fig. 5A, E, I, M), characterized by five small central teeth, five rows of lateral teeth, and two rows of marginal teeth. Counting the teeth of the radula from SEM pictures, the central/rachidian teeth are symmetrical and trapezoid in shape, lateral teeth are claw-like alternately long and short denticles; *G. quinquelirata* sp. nov. has 9–13 denticles, *G. kohsichangensis* sp. nov. has 7–11 denticles, *G. sagitta* sp. nov., and *G. digitinota* sp. nov. have 7–13 denticles, each row of lateral teeth decreasing in size from the first lat-

eral tooth toward the 5th lateral tooth and marginal teeth are claw-like equal-sized denticles; *G. quinquelirata* sp. nov., *G. sagitta* sp. nov. and *G. digitinota* sp. nov. have 12–14 denticles, *G. kohsichangensis* sp. nov. has 14–18 denticles. Therefore, the radula formula is 2:5:1+1+1+1+1+1:5:2. In describing the radula's central tooth formula, the notation reflects the variation in the appearance and orientation of each tooth. In our case, teeth 1–2 curve to the right, tooth 3 is normal, and teeth 4–5 curve to the left. This notation helps communicate the structure and asymmetry of the radula. However, it is noteworthy that variations exist among specimens in terms of the length or curvature of the teeth. The four newly discovered species are described below in the phylogenetic analysis section.

Phylogenetic analysis

The phylogenetic analysis is based on 29 sequences from 10 species of *Georissa*, including the four new species described herein. Sequences of *Diplommatina centralis* and *Plectostoma concinnum* were used as outgroups to root the tree. All sequences were deposited in GenBank (Table 1). The COI alignment had a length of 600 base pairs. Based on the outcomes from jModelTest 2, the best model selection for constructing a COI gene phylogenetic tree according to the BIC is the General Time Reversible (GTR) model. This model incorporates rate variation across sites using a gamma distribution (G) with the proportion of invariable sites (I). The phylogenetic tree revealed *Georissa* as monophyletic. The new *Georissa* species form two distinct clades. *Georissa quinquelirata* sp. nov., *G. kohsichangensis* sp. nov., and *G. sagitta* sp. nov. are a monophyletic and paraphyletic group with *G. digitinota* sp. nov. *Georissa digitinota* sp. nov. is the sister taxon of the *Georissa* species from Malaysian Borneo (Khalik et al. 2018, 2019a, 2019b) (refer to Fig. 3).

Systematics

Family Hydrocenidae Troschel, 1857

Subfamily Neritimorpha Koken, 1896

Genus *Georissa* Blanford, 1864

Type species. *Georissa pyxis*, Benson 1856, by original designation.

Georissa quinquelirata Klongkaew, Poeaim & Dumrongrojwattana, sp. nov.

https://zoobank.org/70D34F82-2195-4F29-89F2-24C53848CF95

Figs 2A, 4A–D, 5A–D

Type material. ***Holotype*** • ZRCBUU 0900 (Fig. 2A). SH = 2.45 mm, SW = 1.63 mm, AH = 0.80 mm, AW = 0.91 mm, W = 4.25–4.5. ***Paratype*** • ZRCBUU 0901 (Fig. 4A–D) (13 shells); Shell measurements: SH = 1.91–2.36 mm (2.10 ± 0.15 mm), SW = 1.37–1.60 mm (1.46 ± 0.07 mm), AH = 0.71–0.86 mm (0.78 ± 0.06 mm), AW = 0.80–0.92 mm (0.86 ± 0.04 mm), W = 3.75–4.5 (all type material from type locality; 25 February 2023, P. Dumrongrojwattana leg).

Type locality. Thailand, Pathawi limestone hill, Thap Than district, Uthai Thani Province; 15°28'26.9"N, 99°45'25.0"E.

Etymology. The specific designation "*quinquelirata*" indicates the number of lirae on the whorl.

Diagnosis. Shell minute, ovately conical, orangish to brownish, protoconch round to slightly ellipsoid and wrinkled sculpture. Body whorl have about 5–6 prominent carinae, and between carinae have weak growth lines. Aperture semi-rounded. Umbilicus closed. Operculum corneous with apophysis, paucispiral, opaque white, and semi-rounded.

Description. Shell minute, dextral, orangish to brownish, ovately conical with 4.25–4.5 whorls (Fig. 2A). Protoconch orange, about one whorl, round to slightly ellipsoid, covered with wrinkled sculpture (Fig. 4B, C). Teleoconch orange, 3.25–3.5 whorls. Body whorl peripherally rounded, sculptured with 5–6 prominent carinae, weak growth lines between carinae, intersected by weak oblique growth lines. Basal part of body whorl with approximately 9–10 densely spaced spiral cords (Figs 2A, 4A). Suture incised. Aperture semi-rounded. Peristome thin, sharp, unexpanded or thickened, not reflected. Umbilicus closed (Figs 2A, 4A). Operculum paucispiral, nucleus submarginal, corneous, opaque white, semi-rounded, thin, smooth outside surface with apophysis, inside surface with an arched peg arising from base (Fig. 4D).

Radula. Ribbon-like, slender, longer, and delicate towards the outside, rhipidoglossate (Fig. 5A–D). Central tooth five small teeth, trapezoid shape. Lateral teeth five teeth, claw-like, with 9–13 alternately long and short denticles decreasing in size from the first lateral tooth toward the 5th lateral tooth. Marginal teeth two teeth, claw-like, with 12–14 equal-sized denticles. Radula formula 2:5:1+1+1+1+1+1:5:2.

Differential diagnosis. *Georissa quinquelirata* sp. nov. resembles *G. hungerfordi* Godwin-Austen, 1889 from Sabah, Borneo, Malaysia, *Georissa hungerfordi* differs in having a less convex body whorl, a smooth sculpture, and more spiral lirae (7–10 lirae instead of 5–6 carinae). This new species is also like *G. liratula* Stolixzka, 1871 from Domotha, Moulmein, but differs in having a more slender shell with fewer spiral lirae.

Georissa kohsichangensis Klongkaew, Poeaim & Dumrongrojwattana, sp. nov.

https://zoobank.org/77F45669-50F9-4FCF-991F-BFC63C7327E7

Figs 2B, 4E–H, 5E–H

Type material. ***Holotype*** • ZRCBUU 0902 (Fig. 2B). SH=2.10mm, SW=1.43mm, AH=0.63mm, AW=0.90mm, W = 4.25–4.5. ***Paratype*** • ZRCBUU 0903 (Fig. 4E–H) (12 shells); Shell measurements: SH = 1.72–2.03 mm

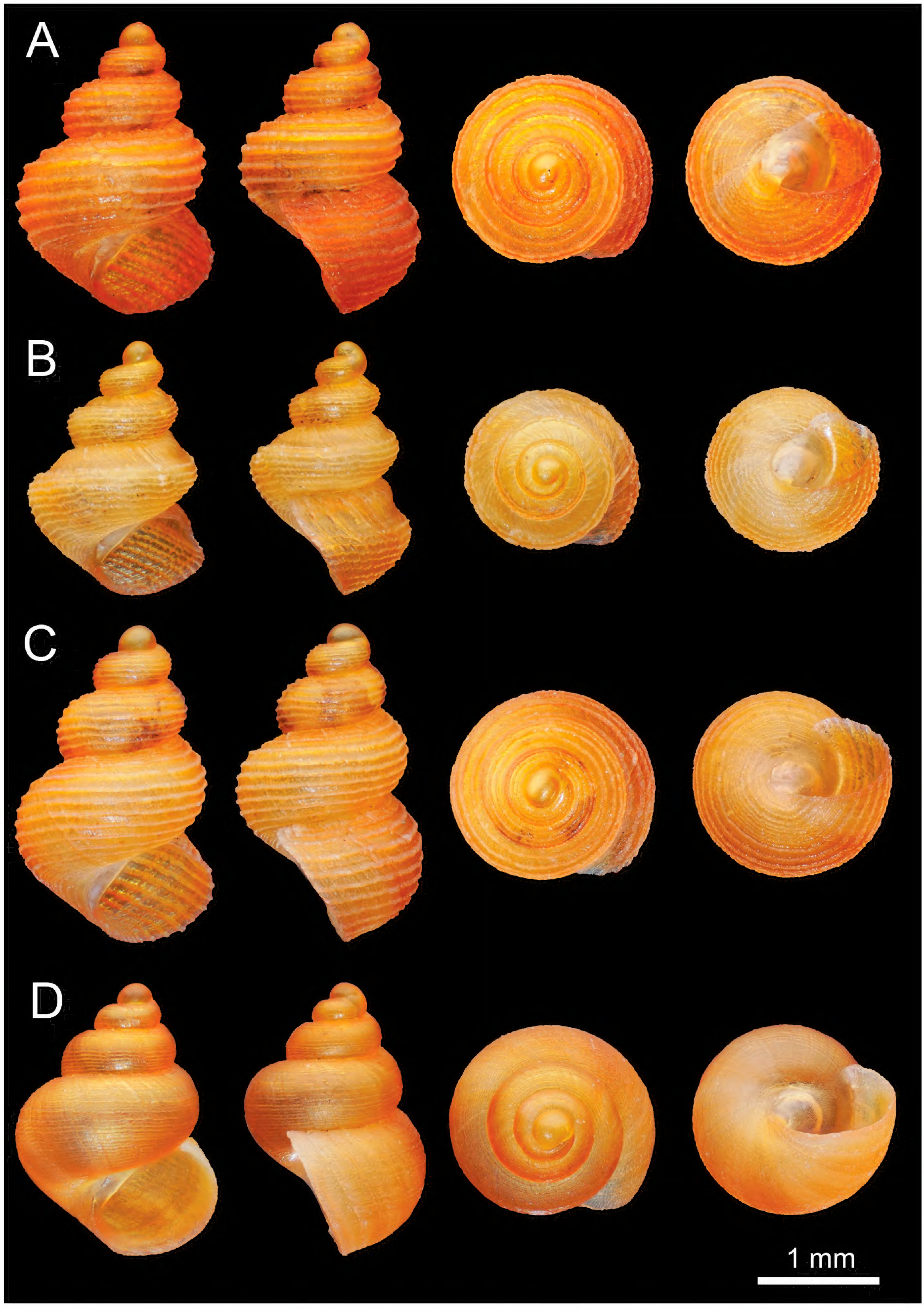

Figure 2. Shell morphology of the new *Georissa* species: **A.** *G. quinquelirata* sp. nov. (Holotype ZRCBUU 0900); **B.** *G. kohsichangensis* sp. nov. (Holotype ZRCBUU 0902); **C.** *G. sagitta* sp. nov. (Holotype ZRCBUU 0904), and **D.** *G. digitinota* sp. nov. (Holotype ZRCBUU 0906).

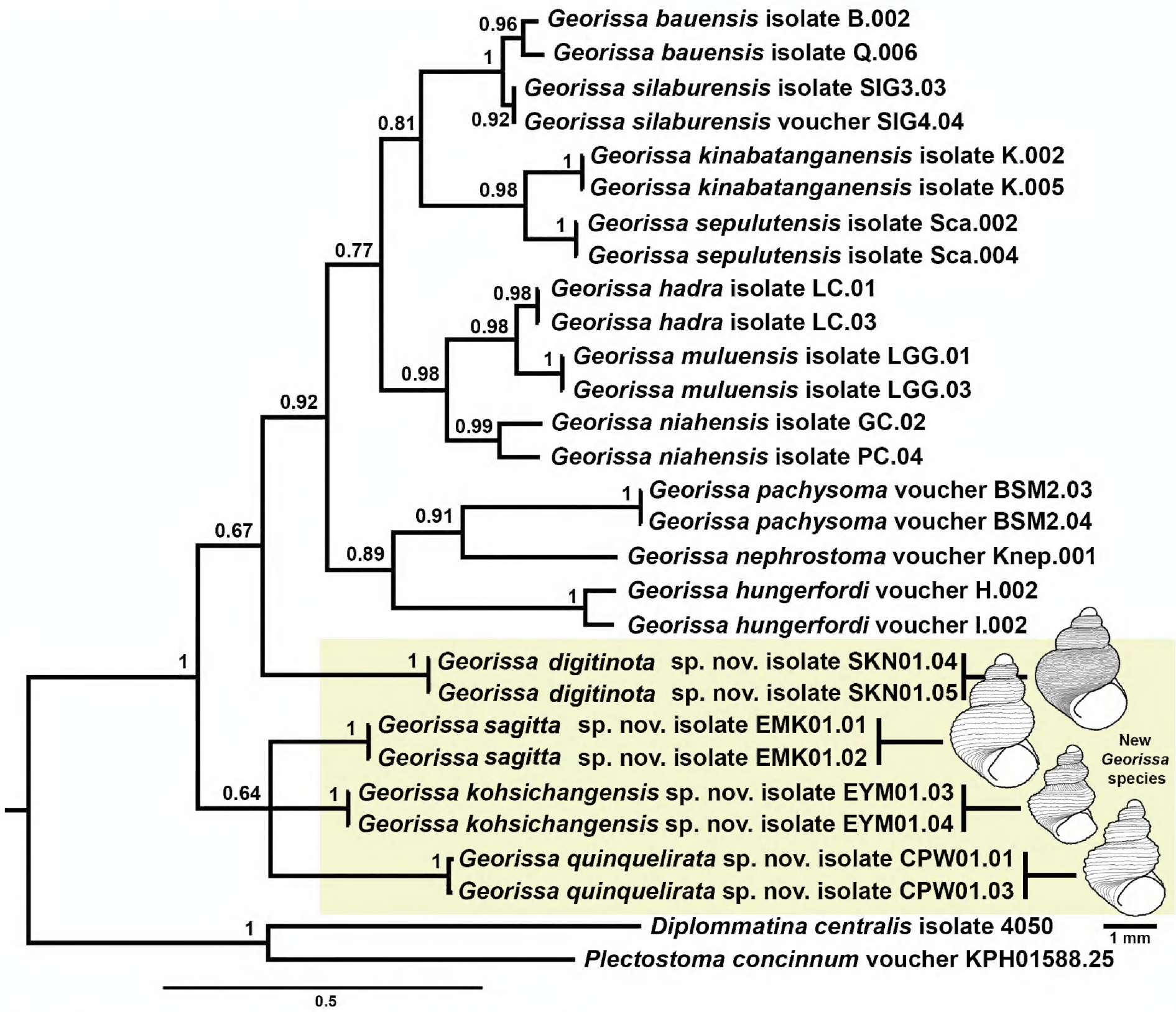

Figure 3. Bayesian inference of phylogenetic analysis (using the GTR+G+I model) with MrBayes for *Georissa*, based on the COI gene. The posterior probability is displayed on each node in the nucleotide sequence.

(1.86 ± 0.10 mm), SW = 1.21–1.42 mm (1.30 ± 0.07 mm), AH = 0.51–0.72 mm (0.62 ± 0.05 mm), AW = 0.75–0.84 mm (0.79 ± 0.03 mm), W = 3.75–4.5 (all type material from type locality; 30 October 2021, P. Dumrongrojwattana leg).

Type locality. Thailand, Yai Man cave, Koh Sichang district, Chonburi Province; 13°09'08.2"N, 100°48'28.0"E.

Etymology. This specific designation "*kohsichangensis*" is a district referring to the type locality.

Diagnosis. Shell minute, ovately conical and the upper half part of the whorls is slightly downward, orangish to brownish, protoconch round to slightly ellipsoid and perforated sculpture. Body whorl with 6–7 spiral lirae. Aperture rhombate. Umbilicus closed. Operculum corneous with apophysis, paucispiral, opaque white, and rhombate.

Description. Shell minute, dextral, orangish to brownish, ovately conical with 4.25–4.5 whorls (Fig. 2B). Protoconch orange, about one whorl, round to slightly ellipsoid, covered with perforated sculpture (Fig. 4F, G). Teleconch orange 3.25–3.5 whorls. The upper half part of the body whorls is slightly downward, sculptured with only strong oblique growth lines, 6–7 prominent lirae, weak oblique growth lines present on the half lower part of the body whorls, more densely spaced spiral cords, ca. 6–8 cords, at the basal part of the body whorl (Fig. 2B, 4E). Suture incised. Aperture rhombate. Peristome thin, sharp, unexpanded or thickened, not reflected. Umbilicus closed. Operculum paucispiral, nucleus submarginal, corneous, opaque white, rhombate shape, thin, smooth outside surface with apophysis, inside surface with an arched peg arising from base (Fig. 4H).

Radula. Ribbon-like, slender, longer and delicate towards the outside, rhipidoglossate (Fig. 5E–H). Central tooth five small teeth, trapezoid shape. Lateral teeth five teeth, claw-like with 7–11 alternately long and short denticles decreasing in size from the first lateral tooth toward the 5th lateral tooth. Marginal teeth two teeth, claw-like with 14–18 equal size denticles. Radula formula 2:5:1+1+1+1+1+1:5:2.

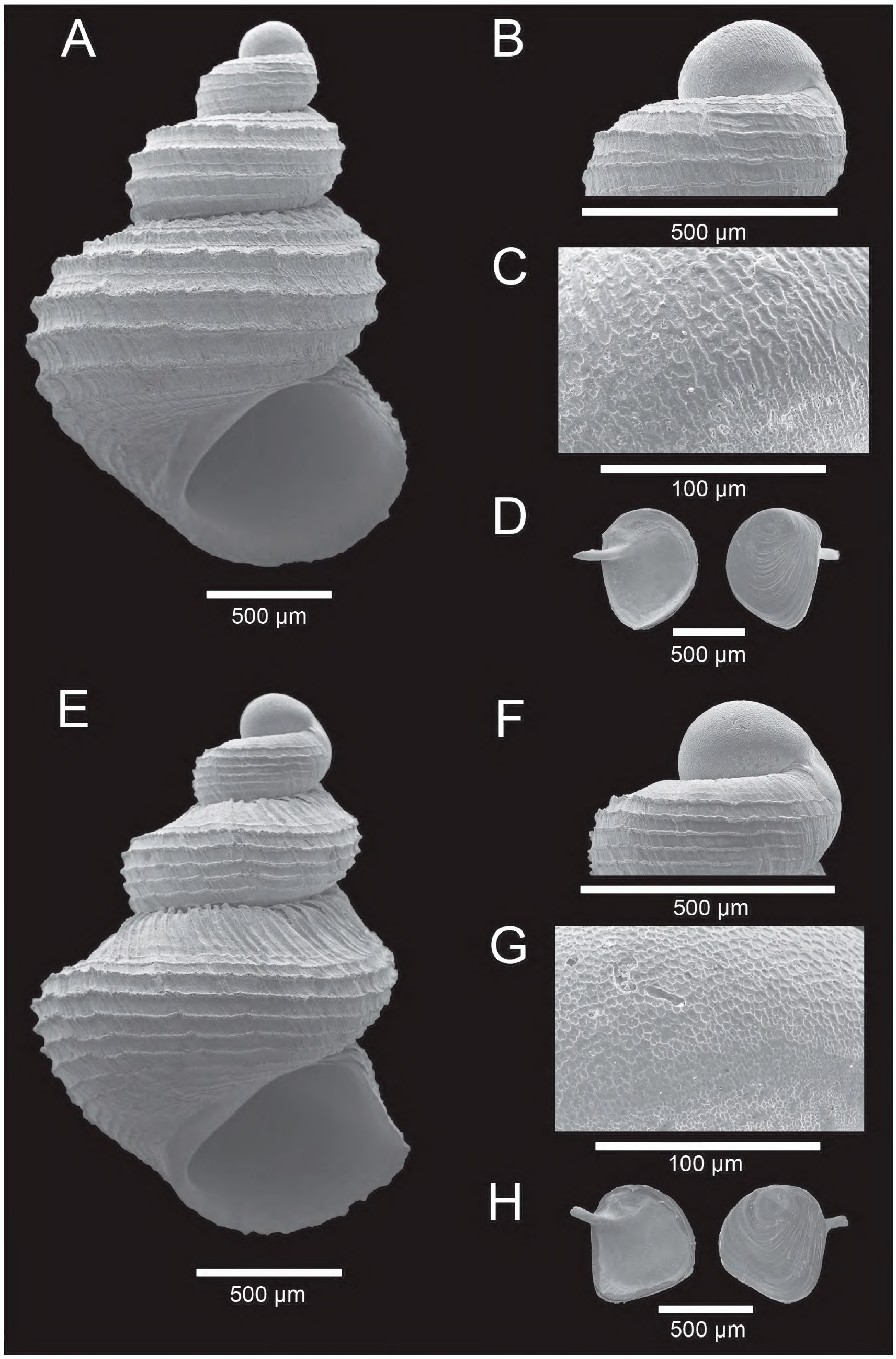

Figure 4. SEM of *Georissa* new species. **A–D.** *G. quinquelirata* sp. nov. (Holotype ZRCBUU 0901); **E–H.** *G. kohsichangensis* sp. nov. (Holotype ZRCBUU 0903); **A, E.** Shell; **B, F.** Protoconch; **C, G.** Sculpture of protoconch; **D, H.** Operculum; **I–L.** *G. sagitta* sp. nov. (Holotype ZRCBUU 0905); **M–P.** *G. digitinota* sp. nov. (Holotype ZRCBUU 0907); **I, M.** Shell; **J, N.** Protoconch; **K, O.** Sculpture of protoconch; **L, P.** Operculum.

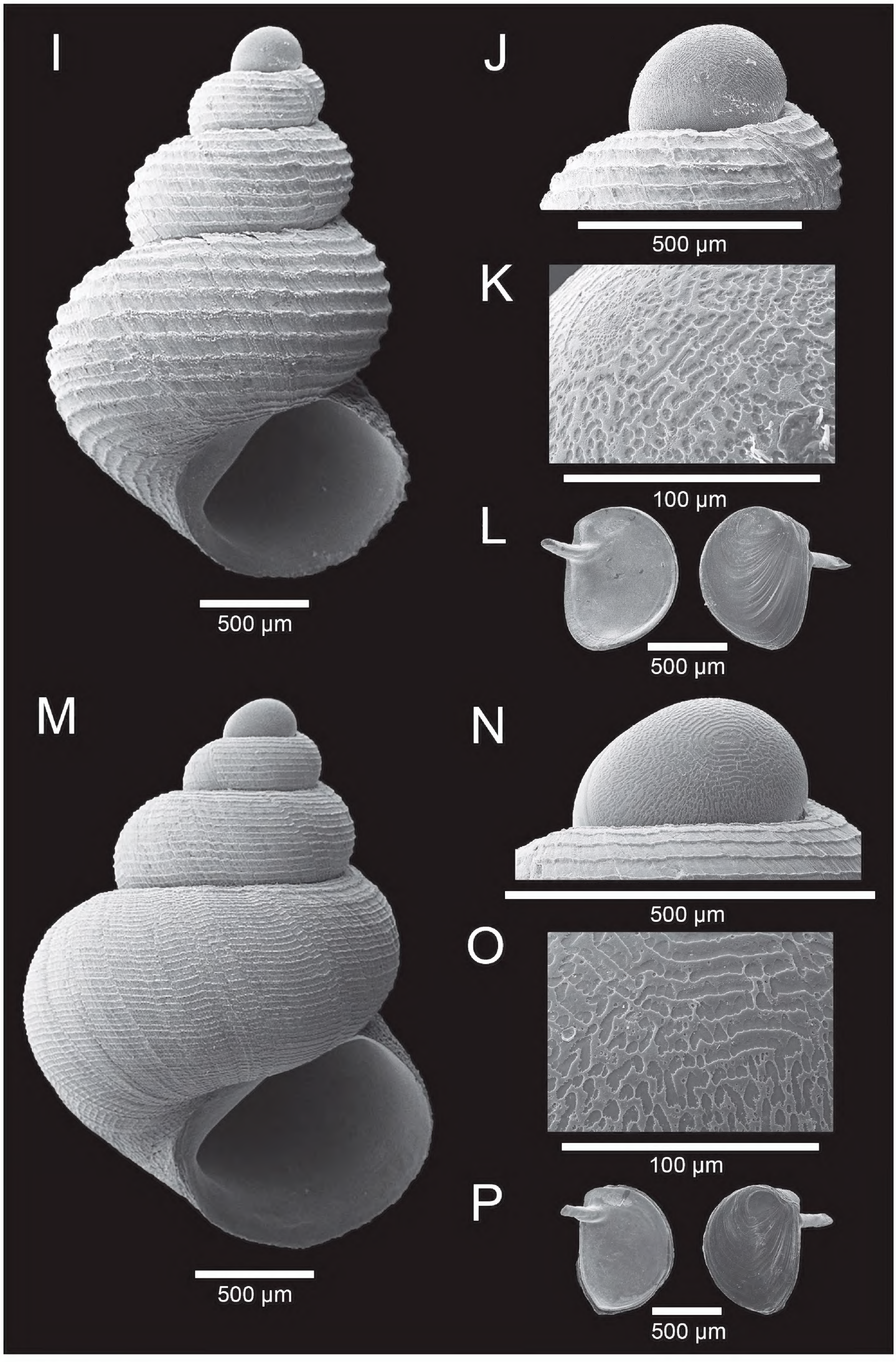

Figure 4. Continued.

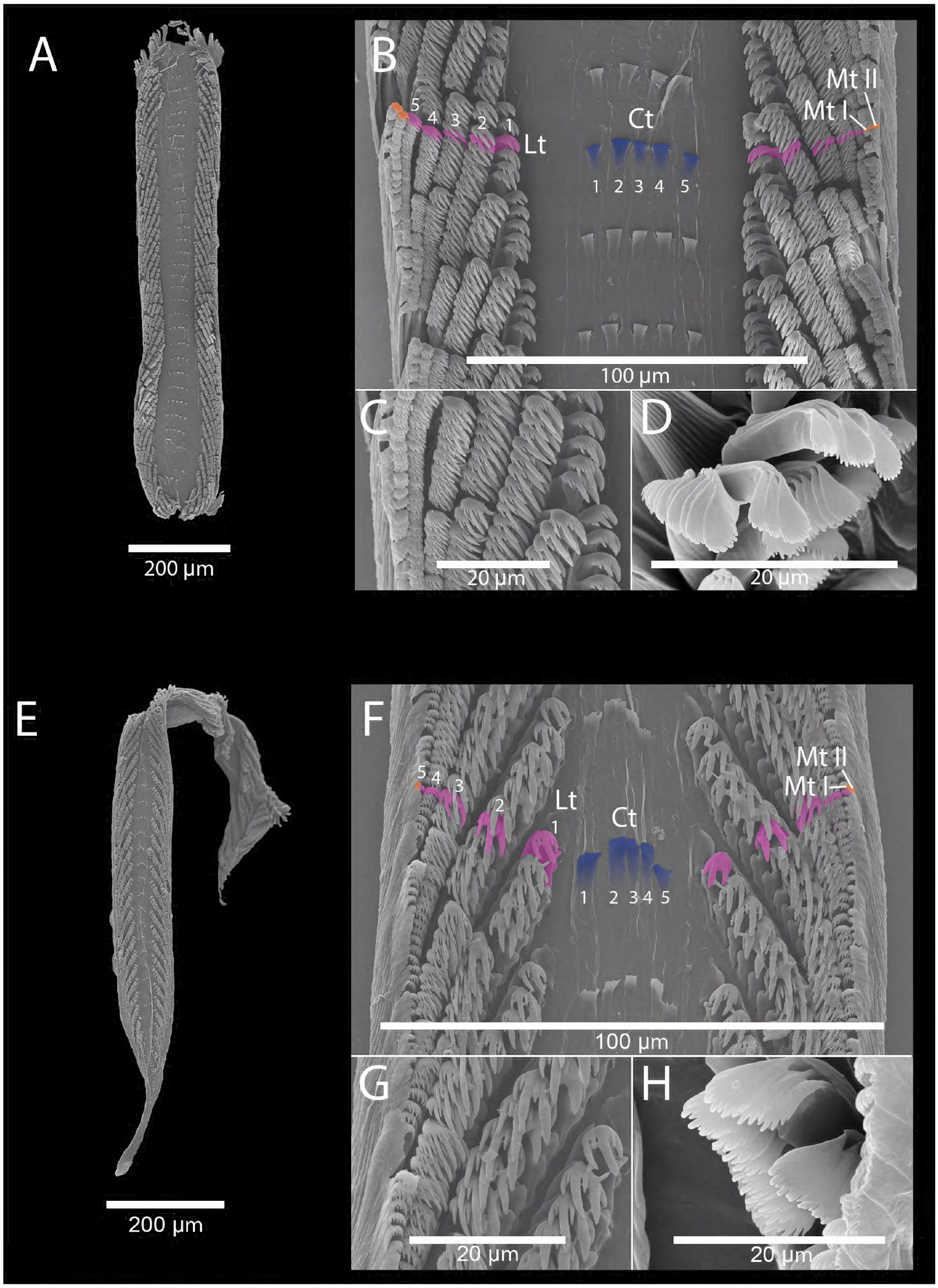

Figure 5. SEM of radula morphology of *Georissa*. **A–D.** *G. quinquelirata* sp. nov.; **E–H.** *G. kohsichangensis* sp. nov.; **A, E.** Radula overview; **B, F.** Radula segment; **C, G.** Lateral teeth (Lt); **D, H.** Marginal teeth (Mt); **I–L.** *G. sagitta* sp. nov. **M–P;** *G. digitinota* sp. nov.; **I, M.** Radula overview; **J, N.** Radula segment; **K, O.** Lateral teeth (Lt); **L, P.** Marginal teeth (Mt). Color highlights show radula position; blue: small central teeth (Ct), purple: lateral teeth, and orange: marginal teeth.

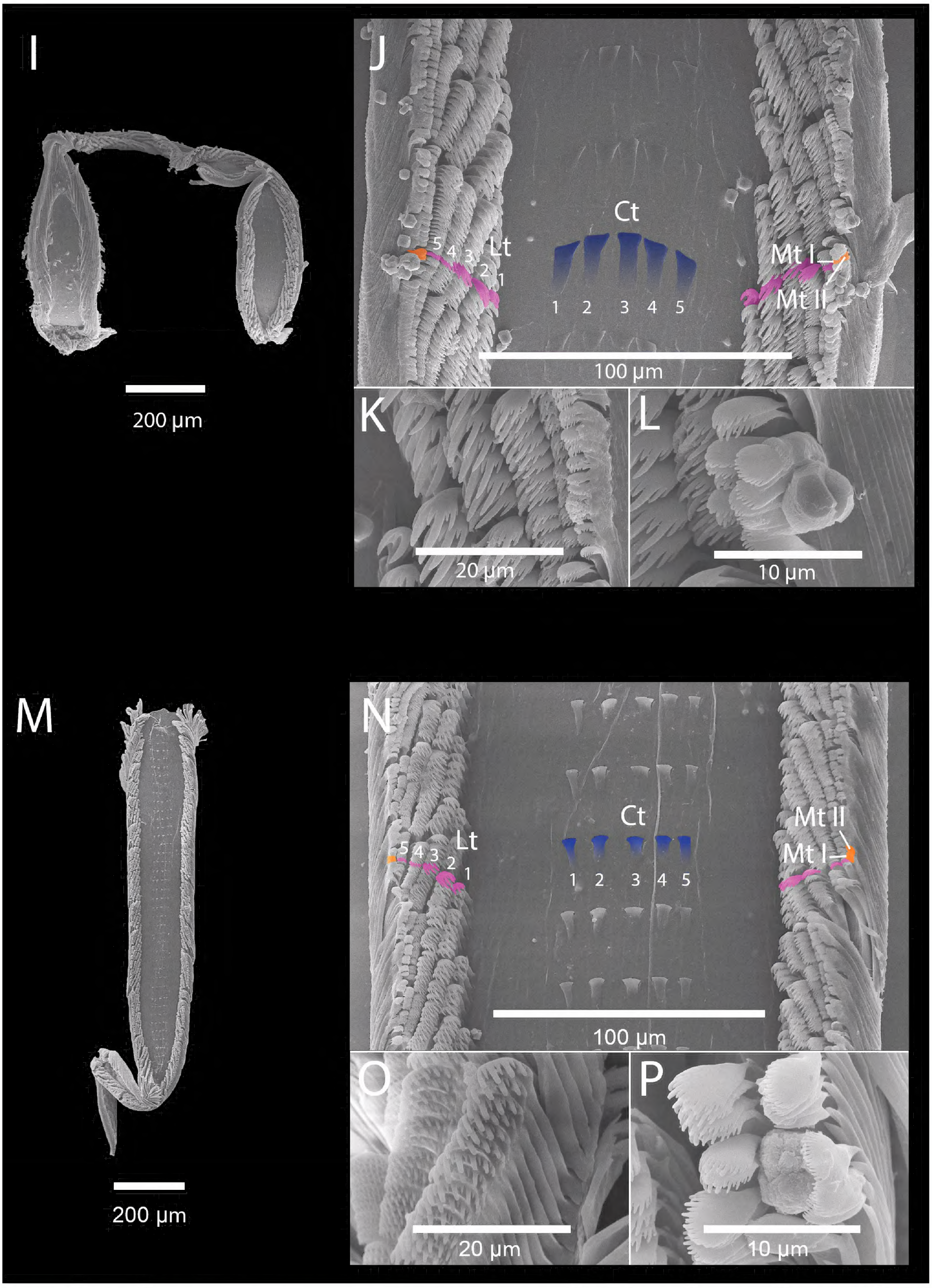

Figure 5. Continued.

Differential diagnosis. *Georissa kohsichangensis* sp. nov. resembles *G. carinata* Sutcharit & Jirapatrasilp, 2020 from Cambodia. *Georissa carinata* differs in having more convex body whorl, a smooth protoconch, and sculptured with thin and uneven growth lines.

Georissa sagitta Klongkaew, Poeaim & Dumrongrojwattana, sp. nov.

https://zoobank.org/965F36E8-E123-496F-A43D-9C2D1B80294F

Figs 2C, 4I–L, 5I–L

Type examined. ***Holotype*** • ZRCBUU 0904 (Fig. 2C); SH = 2.61 mm, SW = 1.63 mm, AH = 0.79 mm, AW = 0.98 mm, W = 4.25–4.5. ***Paratypes*** • ZRCBUU 0905 (Fig. 4I–L) (10 shells); Shell measurements: SH = 2.44–2.88 mm (2.65 ± 0.14 mm), SW = 1.63–1.84 mm (1.70 ± 0.07 mm), AH = 0.79–0.97 mm (0.87 ± 0.05 mm), AW = 0.95–1.09 mm (1.00 ± 0.04 mm), W = 4–4.5 (all type material from type locality; 20 July 2019, P. Dumrongrojwattana leg).

Type locality. Thailand, Khao Maka Cave Temple, Mueang Sakaeo district, Sakaeo Province; 13°47'16.6"N, 101°56'53.7"E.

Etymology. The specific designation "sagitta" for the prominent oval indentation resembling a target in archery.

Diagnosis. Shell minute, ovately conical, orangish, protoconch round to slightly ellipsoid and mix of rounded, ellipsoidal to irregular sculpture with oval indentation. Body whorl peripherally rounded with 8–9 spiral lirae. Aperture semi-rounded. Umbilicus closed. Operculum corneous with apophysis, paucispiral, opaque white, and semi-rounded.

Description. Shell minute, dextral, orangish to brownish, ovately conical with 4.25–4.5 whorls (Fig. 2C). Protoconch orange consists of 1 whorl, round to slightly ellipsoid, covered with mix of rounded, ellipsoidal to irregular sculpture and a large oval indentation present (Figs 4J, 4K). Teleconch orange, consists of 3.25–3.5 whorls. Peripherally rounded, sculptured with 8–9 prominent lirae on the body whorl, crossed with weak oblique growth lines. Basal part of the body whorl, there are more densely spaced spiral cords, approximately 7–8 cords (Figs 2C, 4I). Suture incised. Aperture semi-rounded. Peristome thin, sharp, unexpanded or thickened, not reflected. Umbilicus closed. Operculum paucispiral, nucleus submarginal, corneous, opaque white, semi-rounded, thin, smooth outside surface with apophysis, inside surface with an arched peg arising from base (Fig. 4L).

Radula. Ribbon-like, slender, longer and delicate towards the outside, rhipidoglossate (Fig. 5I–L). Central tooth five small teeth, trapezoid shape. Lateral teeth five teeth, claw-like with 7–13 alternately long and short denticles decreasing in size from the first lateral tooth toward the 5th lateral tooth. Marginal teeth two teeth, claw-like with 12–14 equal size denticles. Radula formula 2:5:1+1+1+1+1+1:5:2.

Differential diagnosis. *Georissa sagitta* sp. nov. resembles *G. monterosatiana* Godwin-Austen & Neville, 1879. *Georissa monterosatiana* differs in having more convex body whorl, smooth protoconch, and more lirae (9–11 instead of 8–9 lirae).

Georissa digitinota Klongkaew, Poeaim & Dumrongrojwattana, sp. nov.

https://zoobank.org/ABD3A8ED-52D0-41C9-85D8-FD5062076E92

Figs 2D, 4M–P, 5M–P

Type examined. ***Holotype*** • ZRCBUU 0906 (Fig. 2D); SH = 2.27 mm, SW = 1.68 mm, AH = 0.85 mm, AW = 1.03 mm, W = 4.25–4.5. ***Paratypes*** • ZRCBUU 0907 (Fig. 4M–P) (9 shells); Shell measurements: SH = 2.34–2.72 mm (2.46 ± 0.12 mm), SW = 1.66–1.86 mm (1.76 ± 0.06 mm), AH = 0.87–1.00 mm (0.93 ± 0.04 mm), AW = 0.96–1.06 mm (1.00 ± 0.04 mm), W = 4–4.5 (all type material from type locality; 24 June 2021, P. Dumrongrojwattana leg).

Type locality. Thailand, Khao Noi Bodhiyan Temple, Mueang Satun district, Satun Province; 06°45'24.2"N, 100°01'54.2"E.

Etymology. The specific designation "digitinota" is for the fingerprints-like sculpture on the protoconch.

Diagnosis. Shell minute, globosely conical, orangish to brownish, protoconch round to slightly ellipsoid and ellipsoidal to irregular sculpture with oval fingerprint-like indentation. Body whorl peripherally rounded with more than 30 spiral lines. Aperture semi-rounded. Umbilicus closed. Operculum corneous with apophysis, paucispiral, opaque white, and semi-rounded.

Description. Shell minute, dextral, orangish to brownish, globosely conical with 4.25–4.5 whorls. Protoconch brownish consists of 1 whorl, round to slightly ellipsoid, covered with ellipsoidal to irregular sculpture and a large oval fingerprint-like indentation present (Fig. 4N, O). Teleconch, orangish to brownish, consists of 3.25–3.5 whorls. Body whorl peripherally rounded, sculptured with more than 30 spiral lines on the body whorl, crossed with very weak oblique growth lines at the basal part of the body whorl (Fig. 4M). Suture incised. Aperture semi-rounded. Peristome thin, sharp, unexpanded or thickened and not reflected. Umbilicus closed. Operculum paucispiral, nucleus submarginal, corneous, opaque white, semi-rounded, thin, smooth outside surface with apophysis, inside surface with an arched peg arising from base (Fig. 4P).

Radula. Ribbon-like, slender, longer and delicate towards the outside, rhipidoglossate (Fig. 5M–P). Central tooth five small teeth, trapezoid shape. Lateral teeth five teeth present, claw-like with 7–13 alternately long and short denticles decreasing in size from the first lateral tooth toward the 5th lateral tooth. Marginal teeth two teeth, claw-like with 12–14 equal-sized denticles. Radula formula 2:5:1+1+1+1+1+1:5: 2.

Differential diagnosis. *Georissa digitinota* sp. nov. resembles *G. monterosatiana* Godwin-Austen & Neville, 1879. *Georissa monterosatiana* differs in having a less convex body whorl, smooth protoconch, and more lirae (9–11 instead of more than 30 spiral lines).

Discussion

The combination of morphological and molecular information supports the distinct separation of the four new *Georissa* species in Thailand; *G. quinquelirata* sp. nov., *G. kohsichangensis* sp. nov., *G. sagitta* sp. nov., and *G. digitinota* sp. nov. However, morphological analysis remains the primary method for distinguishing species. It necessitates the use of specialized equipment for collection and detailed analysis. Their tiny size (1.00–3.00 mm) as *Georissa* in Khalik et al. (2018, 2019b), makes them difficult to detect, collect, and observe, necessitating specialized equipment and techniques.

Several key morphological characteristics are considered. The criteria for classifying each new *Georissa* species based on conchological variability are focusing on shell shape, shell size of height in mm, sculpture pattern of the last (or body) whorl with the number of lines, protoconch sculpture, operculum which has apophysis in ventral view as Neritimorpha snail (Sands et al. 2020), and radula features respectively. The radula teeth of the genus *Georissa* are rhipidoglossate, the radula formula is 2:5:1+1+1+1+1:5:2 and exhibit very similar characteristics. The only notable differences are the number and size of the teeth, which correspond to the size of the *Georissa* snail (Fig. 5). Radula morphology was studied in a research report by Haase and Schilthuizen (2007), focusing on *G. filiasaulae*, a newly discovered species collected from inside a limestone cave in Malaysian Borneo. In their study, they noted the absence of central teeth. However, they encountered difficulty locating these teeth, as they were found only on one side or the other at the time of their study. This is similar to the radula of land micro snails of the genus *Hydrocena*, belonging to the same family, which possesses three small central teeth with a formula represented as ∞1: (1+1+1): 1: ∞ (Egorov 2005).

Molecular analyses were used for the taxon identified and supported morphological evidence such as DNA barcoding and phylogenetic tree (e.g. Khalik et al. 2018, 2019a, 2019b). The phylogenetic analysis indicates that one species, *G. digitinota* sp. nov., bears similarities to other *Georissa* species from Malaysian Borneo due to its similar shape. However, three other species, namely *G. quinquelirata* sp. nov., *G. kohsichangensis* sp. nov., and *G. sagitta* sp. nov., although sharing a similar shape, exhibit slight differences in teleconch sculpture, the number of lirae on the body whorl, and operculum shape. *G. kohsichangensis* sp. nov. has previously been found on Sichang Island and Rin Island in Chonburi Province, which are about 50–60 kilometers on the Google map. Still, this species remains unnamed in the scientific literature (Dumrongrojwattana et al. 2017). When regions are geographically separated, with no spread across limestone hills, it often leads to differentiation between populations in different regions, as isolated groups evolve independently. Additionally, various factors such as historical events, climate, migration patterns, and human activities can influence geographic distribution patterns. Further studies should encompass a broader geographical area and include molecular analysis of Thailand's terrestrial snails, the genus *Georissa*.

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Supplementary material 1

A list of *Georissa* species and their geographical distribution across the mainland in Southeast Asia and China

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Data type: docx

Link: https://doi.org/10.3897/zse.100.128717.suppl1